Alaska Department of Fish and Game
Division of Wildlife Conservation
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Kuiu Island Black Bear Population Estimation Using Biomarking and DNA

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Research Performance Report
1 July 2002–30 June 2003
Federal Aid in Wildlife Restoration
Grant W-33-1, Project 17.7

This is a progress report on continuing research. Information may be refined at a later date.

FEDERAL AID
ANNUAL RESEARCH PERFORMANCE REPORT

ALASKA DEPARTMENT OF FISH AND GAME
DIVISION OF WILDLIFE CONSERVATION
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PROJECT TITLE: Kuiu Island black bear population estimation using biomarking and DNA

PRINCIPAL INVESTIGATOR: Elizabeth Peacock

COOPERATORS: University of Nevada Reno

FEDERAL AID GRANT PROGRAM: Wildlife Restoration

GRANT AND SEGMENT NR.: W-33-1

PROJECT NR.: 17.7

WORK LOCATION: Kuiu Island

STATE: Alaska

PERIOD: 1 July 2002 – 30 June 2003

I. PROGRESS ON PROJECT OBJECTIVES

This document reports on progress for the five objectives of the Kuiu Island Black Bear Project. During this period both field and laboratory studies were undertaken. Field studies took place from May – September 2002 on Kuiu Island. The remainder of the project took place in the conservation genetic facilities at the University of Nevada – Reno.

OBJECTIVES

I. Develop and maintain cooperative agreements and foster interagency and university logistics to conduct field and laboratory studies.

II. Conduct a North Kuiu Island tetracycline biomarking field project in 2002.

III. Estimate black bear use of salmon streams using DNA methods.

IV. Use genetic modeling methods to evaluate effective population size.

V. Assess phylogeography and relative among-island movement rates of bears using molecular genetic models.

II. SUMMARY OF WORK COMPLETED ON JOBS IDENTIFIED IN ANNUAL PLAN THIS PERIOD

OBJECTIVE 1: Develop and maintain cooperative agreements and foster interagency and university logistics to conduct field and laboratory studies.

In the summer of 2002, interagency logistics were coordinated with the USDA Forest Service, Tongass National Forest Petersburg Ranger District office. The DWC Regional Supervisor met multiple times with the District Ranger to ensure that the Forest Service was continuing to support the project. District staff contributed significant resources to the project including access and use of their Kuiu Island facilities at Rowan Bay for the summer, vehicle use, aircraft charter and logistic assistance, housing in Petersburg, and up to 6 staff to help during peak periods of intensive field work. Field studies would not have been possible without this continued support.

A revised cooperative agreement was established with the University of Nevada – Reno in the spring of 2003. This agreement provides support through the spring of 2004 to complete the project. Supplemental funding is being provided via this agreement in support of the graduate student stipend and laboratory costs associated with the increased number of tissue (hair and bone) samples that were acquired.

OBJECTIVE 2: Conduct a North Kuiu Island tetracycline biomarking field project in 2002.

During the previous reporting period we determined that it was more feasible in terms of costs and labor and more beneficial, in terms sample size, to conduct an intensive North Kuiu study as opposed to a baiting study on the entire island.

During this reporting period we completed the field portion of a tetracycline biomarking project (Garshelis and Visser 1997) to estimate the density of black bears (*Ursus americanus*) on North Kuiu Island. Refer to earlier reports (2000, 2001 and 2002) for specific methodology regarding tetracycline baiting, retrieval, bone sampling and analysis. Here we report on progress on this objective for this reporting period.

A total of 263 baits were distributed over four days from 28 June – 1 July by a team of 18 Alaska Department of Fish and Game (ADF&G) employees, United States Forest Service (USFS) employees, and volunteers. Baits were first checked from July 5–8 and were left out if they were not taken, due to low initial bait visitation. Initially, only 53% of the baits had been taken by the first retrieval period. This low bait visitation could be due to (1) difference in scent used (2) lower ambient temperature causing the baits to not to become pungent or (3) a late spring causing a change in habitat use by the bears. Baits were rebaited with meat and molasses. By the end of August, 72–76% of the baits were taken (n = 191–201); this range incorporates ten baits, which may or may not have resulted in a marked bear. A total of 105 toe and tooth samples for tetracycline analysis were collected in the fall 2002 (n = 18) and spring 2003 (n = 87) hunting seasons from Kuiu Island black bear hunters. If a bone or tooth for tetracycline analysis was not collected, the premolar pulled for age analysis will be screened for tetracycline. In addition, samples from western Kupreanof Island were also collected to assess emigration of marked

bears. In fall 2002, 9 tetracycline samples were collected, and 46 samples from spring 2003. The teeth pulled for aging from bears harvested from the remainder of Kupreanof Island will be also screened for tetracycline (n = 32). Preliminary population estimates for North Kuiu Island are expected in the fall of 2003. The simplified basis for the population estimate will be the proportion of bears marked in the hunted sample. This proportion is assumed to be equal to ratio of the number of bears marked (191–200) to the total number of animals in the population. Complexities will be incorporated into the model including proportion of bears emigrating, the year in which the bear was marked (2000 or 2002), and proportion of expected double marking.

OBJECTIVE 3: Estimate black bear use of salmon streams using DNA methods.

Introduction

This objective was designed to test the feasibility of using genetic tagging to estimate the number of black bears (*Ursus americanus*) using stretches of spawning streams used by salmon (*Oncorhynchus keta* and *O. gorbusha*) on North Kuiu Island (56° 35' N, 134° 00' W) in Southeast Alaska (Figure 1). During this period we analyzed DNA bear hair samples collected in 2000.

Genetic tagging (Woods *et al.* 1999) is an increasingly common method of population estimation which involves non-invasive collection, in this case, of hair samples from barbed-wire fences. The genetic identity of these samples is determined and used to establish groups of samples which represent individual animals. With this information, we use the pattern of the capture of individuals to estimate the number of unique bears using each study stream.

Overview of Methods

In 2000, 825 hair samples were collected over the course of the salmon spawning season on five streams on North Kuiu Island (Table 1). Samples were collected from barbed wire fences (n = 107), during 5–8 sessions, each separated by six days. Samples were then dried and stored in paper coin envelopes.

Table 1. Field Effort Data.

Creek	Length surveyed (km)	Density of snares per km	Duration of sampling (days)	# of samples collected
Saginaw	1.8	5.33	42	346
Security	1.6	10.56	43	207
Kadake	3.1*	9.45	37	119
Portage	0.3†	46.15	31	66
Cabin	0.3††	43.75	25	87

*over the course of 11.5 km
† entire spawning course
†† only tidal area

DNA was extracted from 621 samples. This represents 75% of the samples collected (n = 825). DNA was extracted from samples with at least five useable follicles, and only from hairs greater than 5 cm in length, to avoid sampling of cubs. In addition, DNA was only extracted from hair samples that were uniform in color, texture and length, to avoid extracting DNA from multiple bears within a sample. These precautions resulted in the elimination of 204 hair samples. Of the 621 samples extracted, 583 (70% of samples collected; 94% of samples extracted) had enough DNA to be used for Polymerase Chain Reaction (PCR). DNA extracts were then amplified at nine polymorphic microsatellite loci (Paetkau and Strobeck 1994) using PCR. A 'genetic identity' or 'DNA fingerprint' is equated with the sample's genotypes at each of these microsatellite loci. This is called a 'multi-locus genotype.'

Because of the nature of PCR, and the low sample quality and quantity, some PCRs failed. In addition, all of the microsatellite loci developed for *Ursus* are dinucleotide repeats; this means that alleles can differ by only two nucleotide bases, and therefore signals can be ambiguous. I therefore employed the following filters to prioritize reamplification of samples:

1. All samples with rare alleles were reamplified at the questionable locus.
2. All samples with ambiguous genotypes were reamplified at the questionable locus.
3. All samples that differed at one locus were reexamined to elucidate scoring errors (Paetkau 2003).
4. If there were no scoring errors in the mismatch pair, both samples at the questionable locus were reamplified to account for any amplification errors (Paetkau 2003).

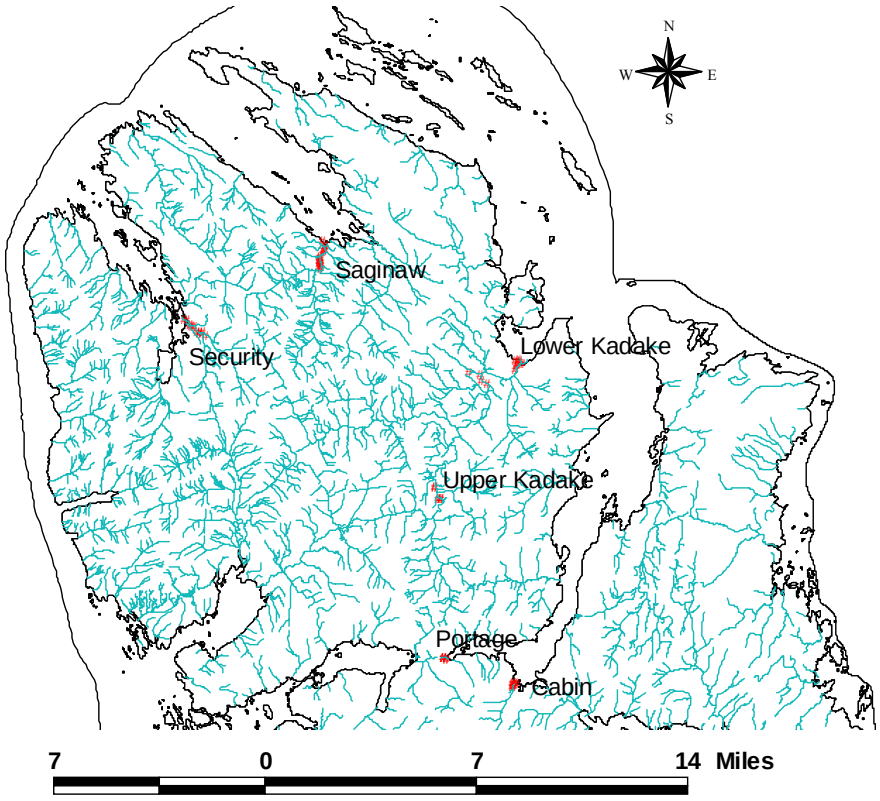

Figure 1. North Kuiu Island. Red marks represent study streams for the genetic tagging project.

Overview of Analyses

I chose to use only samples (n = 446) in which five loci were successfully amplified (75% of those samples amplified, Table 3), given the calculated probability of identity (PI) for North Kuiu Island black bear population. PI (Waits *et al.* 2001), is the probability that a multilocus genotype is shared by two bears. This probability should be as low as possible to correctly identify samples, and decreases with increasing numbers of loci. PI was calculated using the program GIMLET (Valiere 2002). The PI calculated using two sets of five loci, the most and least descriptive loci, is less than 0.01, low enough for individual identification (Mills *et al.* 2000) (Table 2). The PI for each genotype in the data set will be made up of any five of the nine loci, and therefore the actual PI should be somewhere between the two extreme values given in Table 2.

Table 2. Probability of Identity. Overall probability of identity (PI) for North Kuiu Island Black Bears.

Loci	PI (theoretical)	PI (unbiased)	PI (sibs)
5 most informative loci	1.27E-05	1.21E-05	1.14E-02
5 least informative loci	3.18E-03	3.10E-03	7.20E-02
all loci	2.43E-07	2.27E-07	1.75E-03

A dBase program was written (Herzog, M.) and used to identify individuals among the genotypes of the 446 samples. This program performed pairwise comparisons between samples at each of the nine loci and identified samples as individuals defined by five loci. This program also identified pairs that were mismatched at one locus. These mismatch pairs were further analyzed for scoring and amplification errors (Paetkau 2003).

Results

Results on individual identification are tabulated in Table 3. For example, of the 191 samples identified as individuals on the 1.8 km of Saginaw Creek in 2000, 130 were unique individuals sampled once, and 31 were recaptured individuals. Table 4 summarizes various metrics regarding relationship between field effort and unique bears identified.

Table 3. Individual Identification. Black bears identified on North Kuiu Island streams in 2000, and analyzed during this reporting period.

Creek	# of samples amplified	# of samples amplified at ≥ 5 loci	# of identified bears	# of individuals recaptured
Saginaw	282 (82% of collected)	191	130	31
Security	133 (64%)	101	84	15
Kadake	84 (71%)	76	58	17
Portage	39 (59%)	33	29	3
Cabin	45 (52%)	42	25	8

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Table 4. Unique bears caught per effort.

Creek	Bears/Length of Stream	Bears/snare density	Unique bears/days & length sampled
Saginaw	72/km	24.3/snare/km	1.72/km*day
Security	52.5/km	7.9/snare/km	1.22/km*day
Kadake	18/km	6.1/snare/km	0.50/km*day
Portage	97/km	0.6/snare/km	2.69/km*day
Cabin	83/km	0.6/snare/km	3.33/km*day

Discussion

This genetic tagging study suggests that recapture probability of individual bears in the riparian areas of salmon streams is low. This low recapture rate could be due to (1) high turnover of individual bears over the course of the salmon run or (2) avoidance of traps after initial capture. I regard the former explanation as more valid based on observational behavioral data from Kuiu Island; bears individually identified from tree platforms were rarely observed more than once. Secondly, there is no evidence that bears avoid barbed wire snares in any of the other *Ursus* genetic tagging studies (Woods *et al.* 1999, Mowat and Strobeck 2000). Table 3 offers only a *minimum known alive* metric for the five study streams.

OBJECTIVE 4: Use genetic modeling methods to evaluate effective population size.

Progress on this objective during this reporting period involved collection of more tissue samples from black bears from Kuiu Island, and neighboring islands. In the future, DNA from these tissues will be extracted, and genetic and statistical analyses will commence.

OBJECTIVE 5: Assess phylogeography and relative among-island movement rates of bears using molecular genetic models.

The first presentation of this phylogeography study was made at the 14th International Conference for Bear Research and Management in July of 2002. Additional black bear tissue samples were acquired from cooperating area biologists and program technicians when hunter-harvested bears were presented for sealing. The focus was on acquiring samples from islands or mainland areas where sample sizes were low.

Literature Cited

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III ADDITIONAL FEDERAL AID-FUNDED WORK NOT DESCRIBED ABOVE THAT WAS ACCOMPLISHED ON THIS PROJECT DURING THIS SEGMENT PERIOD

None.

IV. PUBLICATIONS

A presentation entitled "Black Bear Wanderings in the Alexander Archipelago" was given at the 14th International Conference on Bear Research and Management in Steinkjer, Norway. This presentation covered preliminary results from objective 5 of this report.

A presentation to the city of Petersburg, Alaska was given entitled "How many bears are there on Kuiu Island." This presentation covered the tetracycline biomarking project and the methods biologists use to count animals.

V RECOMMENDATIONS FOR THIS PROJECT

None at this time.

VI. APPENDIX

VII. PROJECT COSTS FOR THIS SEGMENT PERIOD

FEDERAL AID SHARE $ 67,800 + STATE SHARE $ 22,600 = TOTAL $ 90,400

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